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INFLUENCE OF ALKALOID CONCENTRATION ON ACCEPTABILITY OF TALL LARKSPUR (*Delphinium* SPP.) TO CATTLE AND SHEEP

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Abstract—Tall larkspur (*Delphinium* spp.) is a serious toxic plant problem on western U.S. ranges. The major toxins in tall larkspur are methyllycaconitine (MLA) and 14-deacetylnudicauline (14-DAN); the sum of both is termed the toxic alkaloid concentration. Toxic alkaloids comprise about 20–50% of the total alkaloid concentration in tall larkspur. Toxic and total alkaloid concentration generally declines with maturity, whereas cattle and sheep consumption of larkspur typically increases with plant maturity. We hypothesized that cattle and sheep consumption of tall larkspur was negatively related to higher concentrations of total or toxic alkaloid. We compared consumption of several collections of dried, ground larkspur and fresh larkspur in a series of trials. In another trial, a crude alkaloid fraction was extracted with ethanol, added to alfalfa hay, and consumption compared to untreated alfalfa hay, alcohol-treated hay, and the essentially alkaloid-free plant residue. In all cases we correlated amounts eaten with total and toxic alkaloid concentration. A grazing trial was also conducted to relate larkspur consumption over time to alkaloid concentrations. Total alkaloid concentrations in dried, whole-plant collections ranged from 9.3 to 38.8 mg/g of dry weight, whereas toxic alkaloid concentrations varied from 0.0 to 7.1 mg/g. In one pen trial, cattle preferred a larkspur collection ($P < 0.01$) that contained no toxic alkaloids but had a high total alkaloid concentration (39 mg/g). There was no correlation ($P > 0.05$), however, between concentrations of total or toxic alkaloids and amount of dry plant consumed in this or any other trial. Conversely, sheep consumption tended to be negatively influenced by total and toxic alkaloid concentration ($P \leq 0.08$). In the trials with extract, cattle preferred the alcohol-treated hay and rejected the alkaloid-free residue ($P < 0.01$), whereas the alkaloid-

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treated hay was of intermediate acceptability. Cattle preferred the alkaloid-treated hay over the alkaloid-free residue, indicating that alkaloids did not deter consumption. Conversely, the alkaloid-treated hay was less preferred than either untreated or alcohol-treated hay, suggesting a negative effect on acceptability. There was no correlation between alkaloid concentration and amount of treated feed eaten. In field trials, the amount of composited, fresh leaves or flowers eaten by cattle was influenced by plant part ($P = 0.04$), but was not related ($P > 0.05$) to alkaloid concentration. Cattle preferred leaves over flowers when offered individual plants differing in phenological stage and/or amount of shade, but alkaloid concentration was not related to consumption. We conclude that knowledge of the concentration of total or toxic alkaloid in tall larkspur will give little or no indication of plant acceptability to cattle. Even though accurate predictions can be made about the potential toxicity of larkspur based on the concentration of toxic alkaloids, predictions about consumption must be based primarily on plant phenology.

Key Words—Plant toxins, palatability, diterpenoid alkaloids, methyllycaconitine, *Delphinium* spp.

INTRODUCTION

Tall larkspur (*Delphinium* spp.) is a serious toxic plant problem on western U.S. ranges. Cattle grazing summer ranges sometimes consume large quantities (>30% of diets) of tall larkspur (Pfister et al., 1988a,b) and are often fatally poisoned as a result. Death losses average 5% and may exceed 15% each year on grazing allotments where larkspur is prevalent.

The major toxins in tall larkspur are the diterpenoid alkaloids methyllycaconitine (MLA) and 14-deacetylnudicauline (14-DAN) (Manner et al., 1993). Collectively, these two alkaloids account for about 90% of the toxicity of tall larkspur (Manners and Panter, unpublished data, 1995). These toxins block acetylcholine receptors in the central and peripheral nervous systems, resulting in muscular paralysis and eventually death (Dobelis et al., 1993). Tall larkspur contains other diterpenoid alkaloids in varying quantities (e.g., deltaline), but these other alkaloids are relatively nontoxic (Manners et al., 1993). Thus, in examining the alkaloid concentration of MLA and 14-DAN, the sum of these two toxic alkaloids (MLA + 14-DAN) are termed the toxic alkaloid concentration, and the combined concentration of all alkaloids are the total alkaloids.

The toxic and total alkaloid concentration in leaves generally declines as plants mature (Ralphs et al. 1988; Pfister et al., 1994a), whereas cattle and sheep consumption usually increases with plant maturity (Pfister et al., 1988a,b; Pfister and Manners, 1991, 1995; Ralphs et al., 1991; Ralphs and Olsen, 1992). Based on this inverse relationship, we hypothesized that toxic or total alkaloid concentration negatively influences the acceptability of tall larkspur to cattle and sheep. Our objective in this study was to test this hypothesis using both dried and fresh tall larkspur plant in a series of pen and field trials.

METHODS AND MATERIALS

Cattle Trials

Dried Plant Pen Trials (Trials 1 and 2). Entire tall larkspur plants (*Delphinium barbeyi* and *D. occidentale*) were harvested at various phenological stages (Table 1), air-dried at 25°C, ground to pass a 2-mm screen in a Wiley mill, and stored in sealed plastic bags at 16°C until use. Larkspur alkaloids are stable indefinitely under these conditions. In trial 1, we used ground larkspur collected during 1989, whereas in trial 2 we used ground larkspur collected over a four-year period. We ground the larkspur in order to offer homogenous plant material to subjects and to ensure that subjects could not select for specific plant parts. In both trials, larkspur material was collected at different stages of maturity and/or locations in order to maximize potential differences in alkaloid concentration, since immature larkspur generally contains the highest concentration of total and toxic alkaloids. Ground plant material was subsampled for alkaloid analysis in trials 1 and 2. We hypothesized that immature larkspur would be less acceptable to cattle than mature plant because of a higher alkaloid concentration.

Four yearling Hereford × Angus heifers (290 kg) were used during the spring of 1992 to evaluate the acceptability of the ground larkspur. Animals were group fed ground (2-mm screen) alfalfa hay for 21 days to habituate them to ground feed. Cattle were individually trained in the test procedure for four days. Each subject was given several opportunities to eat small amounts (about 100 g) of rolled barley, and later ground alfalfa, from four feed pans evenly spaced along the fence on one side of the pen. The 36- × 18- × 15-cm (length × width × depth) test pans were placed in specially constructed frames that hooked into the fence in the animal's pen. Animals had free access to all pans for 5 min. The subjects quickly learned to move from pan to pan, eating the small amount of feed in each pan. During testing, animals were fasted until mid-morning when they were tested; they were fed their basal ration of alfalfa hay at midday.

We determined in pilot tests that cattle could consume about 300–500 g of ground alfalfa hay during 5-min test periods. In the test procedure, about 500 g of larkspur material was weighed into each pan, and after an overnight fast, individual animals were offered all four choices simultaneously in the pans for 5 min. Each day the larkspur collections were allocated to a different position in the pen, and each test was run for either three or four consecutive days. Cattle must ingest 8–11 mg of toxic alkaloids per kilogram body weight within a couple of hours for subclinical toxicosis to occur (Pfister et al., 1994b), and animals did not eat enough larkspur to approach this toxic threshold.

Multiple 4 × 4 Latin squares were used initially to assess preference for four different larkspur collections (Borman et al., 1991). Each animal in a pen

TABLE 1. LARKSPUR SPECIES OR FEED TYPE, PHENOLOGICAL STAGE, HARVEST DATE, LOCATION OF PLANT COLLECTION, AND PHYSICAL FORM OFFERED TO CATTLE OR SHEEP DURING ACCEPTABILITY TRIALS.

Species or feed	Phenological stage	Harvest date	Location	Form offered
Trial 1: Cattle and sheep				
Delphinium occidentale	vegetative	6/21/1989	Oakley, ID	air-dried, ground
Delphinium occidentale	flower	7/18/1989	Oakley, ID	air-dried, ground
Delphinium barbeyi	vegetative	7/1/1989	Manti, UT	air-dried, ground
Delphinium barbeyi	flower	8/15/1989	Manti, UT	air-dried, ground
Trial 2				
Delphinium barbeyi	pod	8/25/1986	Manti, UT	air-dried, ground
Delphinium barbeyi	flower	8/12/1987	Manti, UT	air-dried, ground
Delphinium barbeyi	vegetative	7/6/1989	Manti, UT	air-dried, ground
Delphinium barbeyi	flower	8/5/1990	Manti, UT	air-dried, ground
Trial 3				
D. barbeyi extracted residue[a]	flower	7/93	Manti, UT	air-dried, ground
D. barbeyi crude extract[b]	flower	7/93	Manti, UT	mixed with ground hay
Alfalfa + EtOH[c]				air-dried, ground hay
Alfalfa untreated				air-dried, ground hay

Trial 4				
Delphinium barbeyi	full flower[d]	7/16/92	Yampa, CO	fresh, composited flowers
Delphinium barbeyi	early flower[e]	7/16/92	Yampa, CO	fresh, composited leaves
Delphinium barbeyi	early flower	7/16/92	Yampa, CO	fresh, composited flowers
Delphinium barbeyi	full flower	7/16/92	Yampa, CO	fresh, composited leaves
Trial 5				
D. barbeyi plant 1	full flower	7/17/92	Yampa, CO	fresh, cut stalks
D. barbeyi plant 2	early flower	7/17/92	Yampa, CO	fresh, cut stalks
D. barbeyi plant 3	full flower	7/17/92	Yampa, CO	fresh, cut stalks
D. barbeyi plant 4	early flower	7/17/92	Yampa, CO	fresh, cut stalks
Trial 6				
D. barbeyi plant 1	full flower (shaded)	8/7/92	Yampa, CO	fresh, cut stalks
D. barbeyi plant 2	full flower (shaded)	8/7/92	Yampa, CO	fresh, cut stalks
D. barbeyi plant 3	full flower (no shade)	8/7/92	Yampa, CO	fresh, cut stalks
D. barbeyi plant 4	full flower (no shade)	8/7/92	Yampa, CO	fresh, cut stalks
Trial 7				
D. barbeyi plant 1	full pod[f] (no shade)	8/19/92	Yampa, CO	fresh, cut stalks
D. barbeyi plant 2	full pod (shaded)	8/19/92	Yampa, CO	fresh, cut stalks
D. barbeyi plant 3	early pod[g] (shaded)	8/19/92	Yampa, CO	fresh, cut stalks
D. barbeyi plant 4	full pod (no shade)	8/19/92	Yampa, CO	fresh, cut stalks

[a] Larkspur plant residue was the material remaining after extraction with ethanol
[b] Crude extract was extracted, tar-like material; the extract was mixed with ground alfalfa hay
[c] Ethanol was mixed with ground alfalfa hay, then hay was air-dried.
[d] Flowering racemes fully elongated; sepals fully opened and conspicuous
[e] Flowering racemes fully elongated; sepals closed- not visible or inconspicuous
[f] Pods fully developed
[g] Pods partially formed; some flower parts visible

constituted a complete Latin square, with factors: animal (i.e., square), four positions within the pen, four days, and four different larkspur collections (Table 1). After the initial 4 × 4 Latin square was completed for trial 1, the plant material with the highest ranking (i.e., most acceptable) was eliminated, and the test was repeated using three randomly selected animals in a 3 × 3 Latin square; this elimination and retesting procedure was not done during trial 2. We retested to determine if acceptability of the remaining choices was consistent with previous trials.

Larkspur Extract Trial (Trial 3). This trial with ground plant tissue was conducted to assess the acceptability of tall larkspur with the alkaloid fraction removed and to determine if this extracted crude fraction was acceptable when added to alfalfa hay. We hypothesized that cattle would accept the alkaloid-free tall larkspur residue and that cattle would not accept the alfalfa hay when mixed with the crude alkaloid-rich extract. Tall larkspur (*D. barbeyi*) plants were collected during August 1993 near Manti, Utah, on the Wasatch Plateau at about 3000 m elevation, dried at 20°C, and ground to pass a 2-mm screen. Plant material (3.3 kg air-dry weight) was exhaustively extracted with 95% ethanol (EtOH) for four days using a large Soxhlet apparatus to remove all alkaloid compounds. The solvent was removed from the crude alkaloid extract in a rotary evaporator. The tarlike crude alkaloid extract was reconstituted with saline at 3.25 ml/g of extract. The crude extract was evenly applied to 3.3 kg of ground (2-mm screen) alfalfa hay, and air-dried at 20°C for four days. This trial had two controls: untreated, ground (2-mm screen) alfalfa hay and ground alfalfa hay treated with 95% EtOH at 0.75 ml/g of hay. The EtOH was evenly mixed with the hay until all the hay was moist, then the EtOH hay was allowed to dry for four days at 20°C. The extracted larkspur plant residue and crude plant extract containing larkspur alkaloids were subsampled for alkaloid analysis.

The same testing protocol was followed in this trial as in the preceding cattle trials. The four test animals were 3-year-old cows (Hereford × Angus cross; 470 kg); animals were not naive to larkspur, and they were habituated to the procedure before testing.

Fresh Plant Trials (Trials 4–7). Because dried, ground larkspur is not the physical form encountered by grazing cattle, we also conducted acceptability trials using fresh plant material. As with the previous trials, we harvested tall larkspur plants at different phenological stages and/or grown in full or partial sunlight; the intent in all plant collections was to simultaneously offer plants with potential differences in total and total toxic alkaloid concentrations. It was not possible to determine the alkaloid concentration of fresh plants before trials began; hence, we selected plants based on two assumptions: (1) plants grown in full sunlight have higher concentrations of total alkaloids compared to larkspur grown in partial shade (Pfister et al., 1988a), and (2) toxic and total alkaloid concentrations will be lower in mature compared to less mature plants. We hypothesized that leaves or flowers from mature larkspur, with lower alkaloid concentrations, would be more acceptable to cattle compared to immature plant material; and, furthermore, that larkspur at the same growth stage grown in partial shade, with presumably lower alkaloid concentrations, would be more acceptable to cattle compared to larkspur grown in full sunlight.

Trials with freshly harvested tall larkspur (*D. barbeyi*) were conducted during July and August 1992 near Yampa, Colorado, using composite plant collections (trial 4, Table 1) or individual plants (trials 5–7, Table 1). The design for trials 4–7 was a 4 × 4 Latin square with each animal comprising a separate square.

Four yearling Hereford × Angus heifers (320 kg) were used as test animals. A portable corral was constructed adjacent to a larkspur-infested site at 2866 m elevation and a 5- × 8-m pen used for these trials. Animals were gentle and habituated to the test procedure before testing began. Testing was done individually in the morning, with cattle grazing during the remainder of the day.

Composite collections of larkspur leaves and flowers were tested as fresh material (trial 4, Table 1). Numerous (i.e., > 15) plants in the same phenological stage were stripped of leaves and flowers on the morning of the first test. Sufficient material was collected to last for all tests. The material was offered in feed pans in different pen locations as noted above. All four choices were offered simultaneously in this and subsequent trials. Between tests on the subsequent three days, the composited collections were sprinkled with water and stored in plastic bags at 10°C. On the last test day the material was not visibly wilted.

In Trials 5–7, individual larkspur plants were cut at ground level and preserved for the remainder of the trials (Table 1). Multiple stalks from the same plant were bound into a single sheaf near the bottom using cord. Plants were offered to cattle after securing them to the pen fence. When not being offered to cattle, individual plants were kept fresh and turgid by placing them under a shaded canopy with the bottom portion of each plant immersed in water. At this high mountain elevation, daytime temperatures are sufficiently cool that wilting did not occur. Each day individual animals were released into a test pen and allowed 5-min access to larkspur. The number of bites by plant part (leaf, stem, or reproductive parts) from each plant was recorded by a single observer. Each day plants were rotated to a new position.

Subsamples (10 g) of fresh flowers and leaves in trial 4 were frozen, freeze-dried, and ground through a 2-mm screen. Alkaloid concentration was determined for individual plants in trials 5, 6, and 7 by harvesting leaves and reproductive parts (i.e., flowers or pods) from two randomly selected stems immediately after the plant was cut; these samples were immediately frozen, then later freeze-dried and ground (2-mm screen) for analysis. The alkaloid concentration of leaves harvested from one or more stems is representative of leaves from the whole-plant (Manners and Pfister, unpublished data).

Lamb Trial

Sheep are not often intoxicated by tall larkspur because they are much less susceptible to the toxic alkaloids. Sheep are sometimes used to graze a pasture before cattle to reduce subsequent larkspur availability to cattle by trampling or consumption (Ralphs et al., 1991). Grazing trials with sheep have shown that sheep are reluctant to eat immature tall larkspur, but will eat mature larkspur (Ralphs et al., 1991, Ralphs and Olsen, 1992); thus, we hypothesized that alkaloid concentration was inversely related to acceptability to sheep. To test this hypothesis, four 6-month-old weaned male lambs were offered ground *D. barbeyi* or *D. occidentale* collected in the vegetative or flower stage (Table 1). We used the same material in this trial as in cattle trial 1. Sheep were naive to larkspur. Lambs were habituated to consuming 1.5 kg/day of ground alfalfa for 21 days. As in the cattle trials (described above), sheep were tested first in a 4 × 4 Latin square, then tested in a 3 × 3 Latin square trial with the most acceptable feed removed. At the end of the 3 × 3 trial, we removed the most acceptable feed, and conducted another trial using a 2 × 2 crossover design with four sheep, two feeds, and two positions over two days.

Cattle Field Grazing Trial

Grazing cattle select leaves from some tall larkspur plants and avoid others; furthermore during some years cattle select large amounts of tall larkspur, whereas during other years cattle may totally ignore larspur in the same location (Pfister et al., 1988a,b; Pfister and Manners, 1991, 1995). We hypothesized that these selection patterns are at least partially mediated by alkaloid concentrations; we predicted that highly selected plant samples would have lower alkaloid concentrations than plants in the general population. We also predicted that consumption of tall larkspur by grazing cattle would show an inverse relationship to total or toxic alkaloid concentration.

Six 1-year-old heifers (302 kg) were grazed from July 15 to August 26, 1992, on a tall larkspur-infested range at 2866 m elevation near Yampa, Colorado. The 8-ha pasture was enclosed with electric fence, and the cattle were allowed to freely graze from 07:00 to 19:00 hr each day. Cattle were not naive to tall larkspur, as they had grazed on similar tall larkspur-infested pastures the previous summer. Tall larkspur was in the early flower stage (i.e., reproductive racemes elongated, but flowers not open) when the trial began. At the end of the trial, all tall larkspur plants were in the pod stage, and some plants were senescing.

Cattle diets were determined using a bite-count procedure (Pfister et al., 1988a,b) on the seven dates that plant samples were collected for alkaloid analysis. Individual subjects were observed for 5-min periods in a predetermined order during all daylight hours when cattle were actively grazing; at the end of the 5-min observation period the observer would observe the next animal in the sequence. We recorded bites of tall larkspur plant parts (flower, pod, or leaf), grasses, other forbs, shrubs, and monkshood (*Aconitum columbianum*). We defined an individual bite as a single cropping motion, always indicated by a head jerk, often acompanied by a visible sweep of the tongue, independent of chewing motions.

Tall larkspur leaves and reproductive parts from >20 plants were collected on seven dates to determine alkaloid concentration as larkspur matured. In addition, on three other occasions cattle were observed eating unusually large amounts of tall larkspur (i.e., >50% of diet for >1 hr), and remaining leaves from several partially consumed plants were collected and designated "palatable larkspur samples." Plant samples were composited, frozen at −20°C, freeze-dried, and ground to pass a 2-mm screen.

Plant Alkaloid Analysis

HPLC Toxic Alkaloid Analysis. One gram of ground, air-dried plant material was extracted in a Soxhlet apparatus with 80% EtOH. Further details of the HPLC procedure are given in Manners and Pfister (1993). Norditerpenoid alkaloids were detected by UV absorbance at 200 and 280 nm.

Total Alkaloid Analysis. Plant samples (100 mg) were weighed into a test tube and extracted with a mixture of $CHCl_3$ (4 ml) and 1 N HCl (4 ml) by mechanical rotation for 45 min. After centrifugation, the aqueous extract was removed and saved, and the plant material was extracted a second time with 1 N HCl (2 ml) for 5 min. The two aqueous extracts were combined and made alkaline with concentrated NH_4OH to pH 9. The samples were then extracted with $CHCl_3$ (2×), and the $CHCl_3$ solution was separated and filtered through Na_2SO_4. The solvent was removed by evaporation at 70°C under a stream of nitrogen. Carbon disulfide (2.0 ml) was added and the samples analyzed by FT-IR spectroscopy for total alkaloids using a quantitative partial least-squares model based on gravimetric analyses of the identically prepared total alkaloid fraction from a set of standard samples (D. Gardner, unpublished data, 1995).

Statistical Analysis

The statistical model for the Latin square trials was: intake = animal in pen (i.e., squares), position in pen, day in pen, larkspur collection or plant (i.e., treatment), error. The protected LSD procedure was used to compare treatment means after a significant F test ($P < 0.05$). Although there were confounding factors (e.g., years and phenology in trial 2; amount of shade in trials 6 and 7), we consider this to be trivial in the analysis because the independent variable of interest was alkaloid concentration. We determined Pearson correlation coefficients (r) relating alkaloid concentrations to: (1) amount eaten (grams) in trials

1-3 (dried, ground material); (2) amount eaten (grams) in trial 4 (composite fresh material); and (3) total bites of leaves, flowers, and pods from individual plants in trials 5-7, with these three trials grouped together for the correlation analysis.

Bite count data from the grazing trial were transformed using an arcsin-square root transformation. The grazing trial was analyzed using ANOVA procedures, with individual animals as blocks and sampling dates as treatments. The GLM procedure of SAS (1987) was used for data analysis. No statistical analysis was possible on corresponding alkaloid concentrations for each date, as composite samples were used and no measure of the variance was available.

RESULTS

Cattle Pen Trials

No differences in consumption due to plant collection were found in trial 1 ($P = 0.13$), although cattle did consume numerically more of the late season *D. barbeyi* and less of the early-season *D. barbeyi* (Table 2). Thus, we rejected the hypothesis that cattle would consume significantly more of the mature larkspur with a lower alkaloid concentration. When the late-season *D. barbeyi* was removed as a choice and the test rerun as a 3×3 Latin square, no differences in consumption were noted ($P = 0.79$) (Table 2). There was no relationship ($P > 0.05$) between total or toxic alkaloid concentrations and the amount of plant eaten (Table 3). There were significant correlations among the specific alkaloid concentrations, with the exception of 14-DAN.

In trial 2, cattle preferred the 1986 *D. barbeyi* collection ($P < 0.01$) over the other plant collections (Table 4). This collection was unusual in that it had no detectable toxic alkaloid concentration, but it did have a high concentration of total alkaloid. Thus, we rejected the hypothesis that total alkaloid concentration negatively influences consumption, but this trial suggested that toxic alkaloid concentration may influence acceptability. There was no correlation ($P > 0.05$), however, between concentrations of total or toxic alkaloids and amount of dry plant consumed in trial 2 (Table 5).

Cattle preferred both EtOH-treated hay and untreated hay during trial 3 (Table 4). The alkaloid-treated hay was of intermediate acceptability, whereas the alkaloid-free residue was rejected. During the 3×3 trial, when the EtOH-treated hay was withdrawn, the ranking remained the same, with cattle preferring untreated alfalfa, consuming moderate amounts of the alkaloid-treated hay, and rejecting the residue (Table 4). We rejected the hypothesis that cattle would preferentially accept the alkaloid-free residue over the alfalfa hay treated with the crude alkaloid extract. We could not reject the hypothesis of alkaloid influences because the alkaloid-treated hay was less preferred than either control

TABLE 2. TOTAL CONSUMPTION DURING TRIAL 1 OF DIFFERENT LARKSPUR COLLECTIONS (WHOLE PLANT), OFFERED AS AIR-DRIED, GROUND MATERIAL TO CATTLE AND SHEEP, AND CONCENTRATION OF METHYLLYCACONITINE (MLA), 14-DEACETYLNUDICAULINE (14-DAN), TOXIC ALKALOID, AND TOTAL ALKALOID IN PLANT MATERIAL

					Concentration (mg/g dry wt)			
Trial/item	Phenological stage[a]	Consumption (g)[b]	$P > F$[c]	$LSD_{0.05}$	MLA	14-DAN	Toxic	Total
Trial 1: 4 × 4, cattle			0.13					
D. occidentale	vegetative	609			6.9	0.2	7.1	35.9
D. occidentale	flower	583			2.3	0.05	2.4	12.2
D. barbeyi	vegetative	367			5.0	0.4	5.4	28.1
D. barbeyi	flower	842			1.2	0.4	1.6	10.2
Trial 1: 3 × 3, cattle			0.79					
D. occidentale	vegetative	545						
D. occidentale	flower	475						
D. barbeyi	vegetative	351						
Trial 1: 4 × 4, lambs			0.01	24.2				
D. occidentale	vegetative	158						
D. occidentale	flower	167						
D. barbeyi	vegatative	136						
D. barbeyi	flower	1048						
Trial 1: 3 × 3, lambs			0.05	34.7				
D. occidentale	vegetative	92						
D. occidentale	flower	294						
D. barbeyi	vegetative	107						
Trial 1: 2 × 2, lambs			0.61					
D. occidentale	vegetative	158						
D. barbeyi	vegetative	109						

[a] Stage of growth when larkspur was collected.
[b] Total consumption by cattle or sheep during Latin square trials.
[c] Probability of a greater F value for a feed (i.e., treatment) effect.

TABLE 3. PEARSON CORRELATION COEFFICIENTS (r) AND PROBABILITIES ($P > |r|$) FOR RELATIONSHIPS AMONG CONCENTRATIONS (mg/g DRY WEIGHT) OF TOTAL ALKALOID TOXIC ALKALOID, METHYLLYCACONITINE (MLA), AND 14-DEACETYLNUDICAULINE (14-DAN), AND AMOUNT OF LARKSPUR EATEN (g) IN TRIAL 1 BY CATTLE ($N = 7$) OR SHEEP ($N = 9$) USING DRIED, GROUND LARKSPUR SAMPLES.

	Total		Toxic		MLA		14-DAN	
	r	$P > r$	r	$P > r$	r	$P > r$	r	$P > r$
Cattle								
Eaten (g)	−0.54	0.45	−0.40	0.36	−0.40	0.37	−0.05	0.91
14-DAN	0.07	0.93	0.21	0.65	0.14	0.77		
MLA	0.99	0.006	0.99	0.0001				
Toxic	0.99	0.0008						
Sheep								
Eaten (g)	−0.62	0.08	−0.64	0.06	−0.66	0.05	0.23	0.55
14-DAN	0.21	0.59	0.18	0.64	0.10	0.80		
MLA	0.99	0.0001	0.99	0.0001				
Toxic	0.99	0.0001						

food. There was, however, no correlation ($P > 0.05$) between any alkaloid concentration and amount consumed (Table 5), but there were significant relationships among all the alkaloid components.

When fresh leaves and flowers were offered to cattle during trial 4, cattle rejected flowers and ate mostly leaves from plants in the full-flower stage ($P = 0.04$) (Table 4). The MLA concentration of the various composite collections varied greatly, but the range of total alkaloid concentrations was relatively small among the four collections. There was a biologically noteworthy, but statistically insignificant, negative relationship between amount of fresh plant eaten and total alkaloid concentration ($r = -0.74$, $P = 0.26$) (Table 5). Unlike the other trials, the only significant correlation among alkaloid concentrations in this trial was between MLA and toxic alkaloids (Table 5).

There were no differences ($P > 0.1$) in acceptability of individual plants in trials 5, 6, or 7 (Table 6). In trials 5 and 6, cattle ate no flowers, but cattle did take some bites of pods during trial 7 (Table 6). There was no correlation between alkaloid concentration and bites taken of plant material (Table 7). In general, all alkaloidal components were significantly correlated with one another. Taking trials 5–7 together, we rejected the hypothesis that mature larkspur would be more acceptable than less mature plant because of lower alkaloid concentrations. We were also unable to detect any significant impact from shade or full sun on amount of leaf material eaten by cattle.

TABLE 4. TOTAL CONSUMPTION BY CATTLE DURING TRIALS 2–4 OF DIFFERENT LARKSPUR COLLECTIONS OR TREATMENTS AND CONCENTRATION OF METHYLLYCACONITINE (MLA), 14-DEACETYLNUDICAULINE (14-DAN), TOXIC ALKALOID, AND TOTAL ALKALOID IN PLANT MATERIAL

					Concentration (mg/g dry wt)			
Trial/item	Phenological stage[a]	Consumption (g)[b]	$P > F$[c]	$LSD_{0.05}$	MLA	14-DAN	Toxic	Total
Trial 2: 4 × 4, Cattle[d]			0.01	73.1				
D. barbeyi	pod, 1986	1234			0.0	0.0	0.0	38.8
D. barbeyi	flower, 1987	355			2.3	0.1	2.4	9.3
D. barbeyi	vegetative, 1989	499			5.8	0.7	6.6	28.2
D. barbeyi	flower, 1990	316			2.7	0.2	3.0	16.4
Trial 3: 4 × 4, Cattle[e]			0.01	66.7				
Alfalfa		1813			0.0	0.0	0.0	0.0
Alfalfa + EtOH		1854			0.0	0.0	0.0	0.0
Larkspur extract + alfalfa		887			1.4	0.2	1.6	11.4
Larkspur plant residue		43			0.0	0.0	0.0	1.1
Trial 3: 3 × 3, cattle			0.01	85.9				
Alfalfa		1189						
Larspur extract + alfalfa		796						
Larkspur plant residue		64						
Trial 4: 4 × 4, cattle[f]			0.04	46.1				
D. barbeyi	leaves–early flower	260			4.7	0.2	4.9	7.4
D. barbeyi	flowers–early flower	20			1.9	0.09	2.0	6.9
D. barbeyi	leaves–full flower	455			2.6	0.09	2.7	5.9
D. barbeyi	flowers–full flower	0			3.4	0.1	3.5	7.6

[a] Stage of growth when larkspur was collected.
[b] Total consumption (g) by cattle or sheep during Latin square trials; air-dried weight for trials 2–4; fresh weight for trial 4.
[c] Probability of a greater F value for a feed (i.e., treatment) effect.
[d] No 3 × 3 trial was conducted.
[e] *D. barbeyi* was extracted with EtOH; the crude extract was added to alfalfa hay; the extracted larkspur residue was also tested; as one control, alfalfa hay had EtOH added, with EtOH allowed to evaporate; untreated alfalfa was also used.
[f] Composite larkspur treatments were made by collecting leaves or flowers from numerous plants in the same phenological stage. Plant was offered fresh and green daily to cattle.

TABLE 5. PEARSON CORRELATION COEFFICIENTS (r) AND PROBABILITIES ($P > |r|$) FOR RELATIONSHIPS AMONG CONCENTRATIONS (mg/g DRY WEIGHT) OF TOTAL ALKALOID, TOXIC ALKALOID, METHYLLYCACONITINE (MLA), AND 14-DEACETYLNUDICAULINE (14-DAN), AND AMOUNT OF LARKSPUR EATEN (g) IN TRIALS 2–4 BY CATTLE (N = 4, 7, and 4, RESPECTIVELY) USING VARIOUS LARKSPUR TREATMENTS[a]

	Total		Toxic		MLA		14-DAN	
	r	$P > r$	r	$P > r$	r	$P > r$	r	$P > r$
Trial 2								
Eaten (g)	0.87	0.12	−0.05	0.95	−0.04	0.96	−0.009	0.99
14-DAN	0.32	0.68	0.99	0.002	0.99	0.002		
MLA	0.31	0.69	0.99	0.0001				
Toxic	0.30	0.69						
Trial 3								
Eaten (g)	−0.18	0.69	−0.12	0.80	−0.12	0.80	−0.10	0.83
14-DAN	0.99	0.0001	0.99	0.0001	0.99	0.0001		
MLA	0.99	0.0001	0.99	0.0001				
Toxic	0.99	0.0001						
Trial 4								
Eaten (g)	−0.74	0.26	0.18	0.81	0.18	0.81	0.24	0.76
14-DAN	0.39	0.60	0.88	0.11	0.86	0.13		
MLA	0.53	0.47	0.99	0.0005				
Toxic	0.53	0.47						

[a] See Table 1.

Sheep Trials

Sheep displayed a distinct preference for the late-season plant material in all three tests. In the 4 × 4 test, they preferred the late-season *D. barbeyi* ($P < 0.01$) over the other three choices (Table 2). In the three-choice test, they also preferred the late season *D. occidentale* ($P = 0.05$) compared to *D. barbeyi* and *D. occidentale* collected in the vegetative stage (Table 2). When only two plant collections were compared in the 2 × 2 crossover tests, lambs showed no preference between *D. barbeyi* and *D. occidentale* in the vegetative stage. Thus, we accepted the hypothesis that total and/or toxic alkaloid concentration negatively influences sheep preference for tall larkspur. There was a strong tendency for sheep preferences to be negatively correlated with total and toxic alkaloid concentrations and to MLA concentration (Table 3). Both MLA and toxic alkaloid concentrations were related to each other and to total alkaloid concentration, but 14-DAN concentration was not related to the other alkaloid components (Table 3).

TABLE 6. CONSUMPTION OF INDIVIDUAL, FRESH *D. barbeyi* PLANTS DURING TRIALS 5–7 AND CONCENTRATION OF METHYLLYCACONITINE (MLA), 14-DEACETYLNUDICAULINE (14-DAN), TOXIC ALKALOID, AND TOTAL ALKALOIDS IN LEAVES AND FLOWERS FROM 2 REPRESENTATIVE STALKS FROM EACH PLANT

Trial/item	Plant phenology/characteristics	Consumption (bites)	$P > F$[a]	Concentration (mg/g dry wt) MLA	14-DAN	Toxic	Total
Trial 5: 4 × 4, cattle			0.60				
Plant 1	Full flower[b]/leaves	134		2.2	0.3	2.5	6.5
Plant 2	Early flower[c]/leaves	117		2.4	0.2	2.6	7.5
Plant 3	Full flower/leaves	251		4.4	0.2	4.6	6.2
Plant 4	Early flower/leaves	92		2.2	0.2	2.6	6.9
			—				
Plant 1	Full flower/flowers	0		2.5	0.2	2.7	5.8
Plant 2	Early flower/flowers	0		1.8	0.1	1.9	5.1
Plant 3	Full flower/flowers	0		2.0	0.1	2.1	6.0
Plant 4	Early flower/flowers	0		2.3	0.1	2.4	6.0
Trial 6: 4 × 4, cattle			0.88				
Plant 1	Full flower–shaded/leaves	182		2.7	0.2	2.9	3.9
Plant 2	Early flower–shaded/leaves	270		2.5	0.1	2.6	6.2
Plant 3	Full flower–no shade/leaves	245		2.7	0.1	2.8	3.6
Plant 4	Early flower–no shade/leaves	270		2.5	0.1	2.7	6.4

TABLE 6. Continued.

Trial/item	Plant phenology/characteristics	Consumption (bites)	$P > F$[a]	Concentration (mg/g dry wt) MLA	14-DAN	Toxic	Total
			—				
Plant 1	Full flower/flowers	0		4.7	0.3	5.0	7.9
Plant 2	Early flower/flowers	0		2.8	0.1	3.0	6.5
Plant 3	Full flower/flowers	0		2.4	0.1	2.6	7.5
Plant 4	Early flower/flowers	0		3.0	0.1	3.1	7.2
Trial 7: 4 × 4, cattle			0.54				
Plant 1	Full pod–no shade/leaves	114		2.1	0.04	2.2	3.1
Plant 2	Full pod–shaded/leaves	236		0.6	0.3	0.9	3.4
Plant 3	Early pod–shaded/leaves	140		1.3	0.1	1.4	4.1
Plant 4	Full pod–no shade/leaves	91		1.4	0.1	1.6	3.8
			0.26				
Plant 1	Full pod–no shade/pod	9		4.8	0.1	5.0	6.9
Plant 2	Full pod–shaded/pod	23		4.1	0.8	5.0	10.2
Plant 3	Early pod–shaded/pod	0		2.0	0.1	2.1	5.8
Plant 4	Full pod–no shade/pod	1		3.9	0.7	4.7	8.4

[a] Probability of a greater F value.
[b] Plants in full flower had fully elongated racemes with flowers fully opened.
[c] Plants in early flower had partially elongated racemes with flowers not fully opened.

TABLE 7. PEARSON CORRELATION COEFFICIENTS (r) AND PROBABILITIES ($P > |r|$) FOR RELATIONSHIPS AMONG CONCENTRATIONS (mg/g DRY WEIGHT) OF TOTAL ALKALOIDS, TOXIC ALKALOID, METHYLLYCACONITINE (MLA), AND 14-DEACETYLNUDICAULINE (14-DAN), AND TOTAL BITES BY CATTLE OF LEAVES AND REPRODUCTIVE PARTS DURING TRIALS 5–7 USING FRESH, CUT INDIVIDUAL LARKSPUR PLANTS ($N = 24$ FOR LEAVES AND REPRODUCTIVE PARTS)

	Total		Toxic		MLA		14-DAN	
	r	$P > r$	r	$P > r$	r	$P > r$	r	$P > r$
Bites	−0.32	0.13	−0.20	0.35	−0.17	0.42	−0.25	0.25
14-DAN	0.65	0.0006	0.53	0.008	0.38	0.06		
MLA	0.64	0.0007	0.98	0.0001				
Toxic	0.72	0.0001						

Cattle Grazing Trial

Cattle consumed 7 ± 2.5% of their overall diet as larkspur during the seven sampling dates. Larkspur consumption differed by dates, as cattle ate little larkspur during July, but increased larkspur consumption during mid- to late August (Figure 1). Most (>90%) of the larkspur consumed was leaves, with flowers and pods being selected only occasionally (data not shown). The concentration of toxic alkaloid in leaves declined slightly in late July and early August, but then increased slightly in mid- to late August (Figure 1). Total alkaloid concentration in leaves peaked in mid-July, decreased during July and much of August, but then increased in late August (Figure 1). On the three occasions (July 19, August 23 and 24) when cattle greatly increased leaf consumption during or just after storms, the toxic and total alkaloid concentration in leaves from preferred plants was 5.8 and 7.6, 1.8 and 5.4, and 2.3 and 6.0 mg/g, respectively, for the three dates. One of these samples contained a substantial amount (>5 mg/g) of toxic alkaloids, and two samples contained low to moderate (~2 mg/g) amounts of toxic alkaloids, and provided circumstantial evidence that toxic alkaloid concentration was not a major factor in selection of tall larkspur by grazing cattle. The leaf concentration of total alkaloids was in the range for most tall larkspur samples collected from this area and did not provide evidence for either avoidance or preference.

DISCUSSION

Our study indicates that concentrations of total or toxic alkaloid in tall larkspur generally have little or no influence on acceptability of tall larkspur to

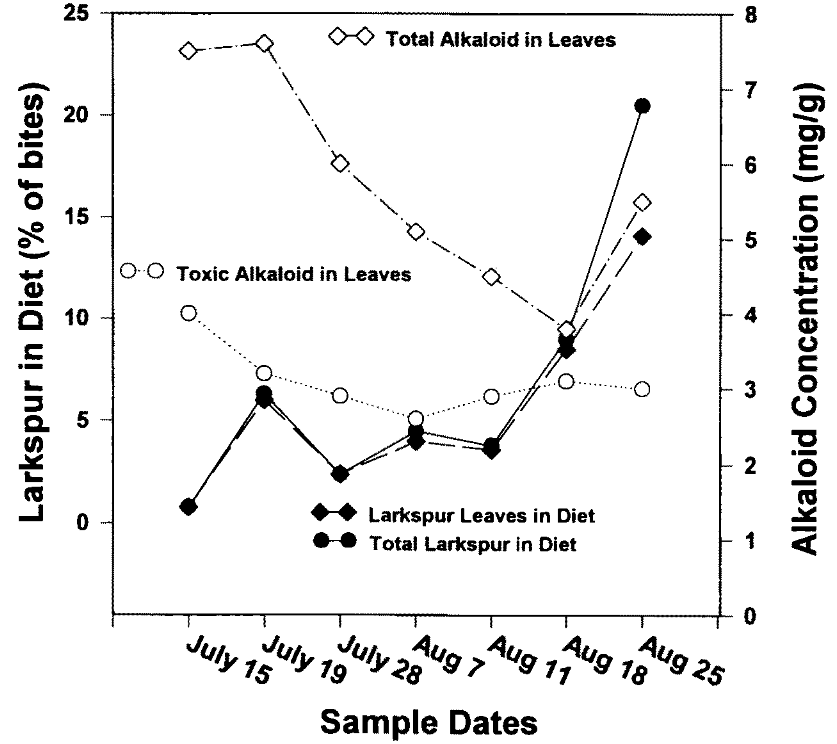

FIG. 1. Mean amounts of tall larkspur in diets of cattle (percentage of bites: leaves and total larkspur) and concentration (mg/g dry weight) of toxic alkaloids and total alkaloids in leaves sampled when cattle diets were determined during a grazing study near Yampa, Colorado, during summer, 1992.

cattle. The pen trials showed few or no relationships between alkaloid concentration (toxic or total) and amounts eaten by cattle, and the field grazing trial also indicated that toxic or total alkaloid concentrations in larkspur do not negatively influence consumption patterns. One collection of *D. barbeyi* from Utah contained no toxic alkaloids, yet had a total alkaloid concentration of nearly 39 mg/g. Cattle preferred this atypical plant collection during pen trials, thus providing strong circumstantial evidence that a high total alkaloid concentration is not deterrent to cattle. Conversely, this result indicates that very low toxic alkaloid concentrations may influence acceptability. Yet, we found no correlation in this or any other trial between toxic alkaloid concentration and amount eaten; further, other collections with lower toxic alkaloid concentrations (<2.0 mg/g) were not differentially accepted by cattle. We cannot explain the increase in total alkaloids in leaves in late August during the grazing trial, but it seems doubtful that cattle increased larkspur consumption in response to this upswing in total alkaloid concentration. Our results are based primarily on correlation; hence, we can suggest causative factors but cannot be definitive about the interpretation. Plant factors other than alkaloids may have influenced our results (Reichardt et al., 1987).

There is disagreement on whether the taste of alkaloids affects palatability. Alkaloids apparently taste bitter to humans (DuPont et al., 1994) and livestock (Laycock, 1978), and bitterness may (Bate-Smith, 1972) or may not (Robinson, 1979; Glendinning, 1994) be universally repellent. Some classes of alkaloids apparently do not have a bitter taste (Molyneux and Ralphs, 1992). The alkaloid concentration of some toxic plants (e.g., *Phalaris* spp., *Lupinus* spp., *Senecio* spp.) is inversely related to palatability or may deter grazing (Ralphs and Olsen, 1987). Other alkaloid-containing toxic plants such as *Oxytropis* spp. and *Astragalus* spp., however, apparently do not show this relationship (Ralphs and Olsen, 1987).

Cattle did not eat enough larkspur in any trial for toxicosis to influence results. Nonetheless, aversive postingestive feedback may limit intake of specific foods (Provenza, 1995), and we have shown that larkspur ingestion by cattle above a toxic threshold is regulated by postingestive consequences (Pfister et al., 1990). Larkspur alkaloidal extracts can cause food aversions (Olsen and Ralphs, 1986), but cattle must consume about 9–11 mg of toxic alkaloids per kilogram of body weight for subclinical toxicosis to influence intake (Pfister et al., 1994b).

For reasons that are unclear, cattle in the pen and grazing trials near Yampa ate few or no flowers or pods. We have noted this same propensity in other grazing trials in this region during three other summers (Pfister, unpublished data). This is in contrast to cattle consumption patterns in Utah, where grazing trials have shown that cattle prefer larkspur pods from mature plants (Pfister et al., 1988a,b). We are presently unable to account for these differences in acceptability.

During trial 3 using alkaloid extract, cattle preferred the larkspur extract on alfalfa hay over the alkaloid-free residue, indicating that alkaloids did not deter consumption. Conversely, the extract-treated hay was less preferred than either untreated or EtOH-treated hay, suggesting a deleterious effect on acceptability. We recognize that extraction may remove many cellular constituents (e.g., some free sugars); however, 95% ethanol extraction only removes a small proportion of the major fructosans (Smith, 1969, 1973). It is possible that cattle selected the alkaloid-treated hay because of carbohydrates that were simultaneously extracted. Alternatively, it is possible that some very nonpolar constituents (e.g., terpenoids) were left in the residue, and acted as feeding deterrents to cattle.

In contrast to cattle, sheep consumption was negatively influenced by concentrations of total alkaloid and MLA in tall larkspur. Sheep apparently have a lower rejection threshold for bitter flavors than do cattle (Goatcher and Church, 1970), and this may account for their negative response to total alkaloid concentration in larkspur. Grazing sheep before cattle in tall larkspur-infested pastures has had limited success (Ralphs et al., 1991) because of the difficulty in getting sheep to eat larkspur before the plant is relatively mature. Our results suggest that higher concentrations of total alkaloid, or MLA, often found in immature larkspur, may be deterrent to sheep.

Arnold and Hill (1972) noted that the total alkaloid concentration of *Phalaris* spp. was more important to palatability than were individual alkaloids. Similarly, we found little indication that individual alkaloids influence cattle consumption; however, MLA concentration was negatively correlated with sheep consumption. Since MLA and total alkaloid concentration were also highly correlated, our sheep trials give no clear indication of the relative importance of total versus MLA alkaloid concentration.

At times there was not a strong correlation between concentrations of total and toxic alkaloids (or MLA concentration). Concentration of MLA was always related to toxic alkaloid concentration, since MLA comprises most of the toxic fraction, but MLA concentration was sometimes not related to amounts of 14-DAN. With plant material collected near Yampa, Colorado (trials 4–7), toxic alkaloid concentration was variable, whereas total alkaloid concentration was relatively low, with ratios of toxic to total alkaloid concentration varying from 0.3 to 0.75. Collections of tall larkspur from either Utah or Idaho (trials 1 and 2) had toxic/total ratios of about 0.2 or less. The extracted residue (trial 3) also did not have any toxic alkaloids, but had a small amount of total alkaloid left after extraction.

Even though we collected both shaded and unshaded larkspur plant for comparisons in trials 6 and 7, no distinct trend emerged for alkaloid concentration as leaves from both shaded and unshaded plants had total alkaloid concentrations averaging about 4 mg/g and toxic alkaloid concentrations averaging about 2 mg/g. The alkaloid concentrations in flowers also did not show clear differences due to shading. We have previously found that grazing cattle prefer to graze shaded larkspur plants over plants growing in sun (Pfister et al., 1988a), but that trend was not evident in this study.

Overall, this study indicates that knowledge of the concentration of total or toxic alkaloid in tall larkspur will not give any clear indication of whether cattle are likely to ingest the plant or not. We can make accurate predictions about the toxicity of tall larkspur based on the concentration of toxic alkaloids, but this information will only provide the livestock producer with the risk of toxicity assuming some level of consumption. Predicted consumption by cattle must necessarily be based on an understanding of other factors, such as plant phenology (Pfister et al., 1988a,b), or weather patterns (Ralphs et al., 1994), but not plant toxicity.

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